

Scientific Highlights at XM-1

W. Meyer-Ilse¹, G. Denbeaux^{1,2}, L.E. Johnson¹, W. Bates¹,
A. Lucero¹, E. H. Anderson¹

¹*Center for X-ray Optics, Lawrence Berkeley National Laboratory, Berkeley California 94720, USA*

²*Physics Department, Duke University, Durham NC 27708, USA*

Abstract

We give an overview of the activities at the high-resolution x-ray microscope XM-1 at the Advanced Light Source, including both scientific programs and instrumental enhancements. The instrument is being actively used in many fields including biology, environmental and material sciences. A new high efficiency condenser zone plate and precision computer control of the microscope allow users to obtain many hundreds of images in a day. Further developments at XM-1 include a cryogenic sample stage for sample preservation and plans for the implementation of a cryo-tilt stage to capture stereoscopic information.

Cryogenic Sample Stage

In order to preserve the structural integrity of biological samples, a cryogenic sample holder has been built. The radiation dose from each exposure at XM-1 is roughly 10^7 Gray which, depending on the sample, can cause changes in the morphology of wet samples after one or two images. In order to keep the samples intact for many images, and also to prevent the formation of ice crystals, the sample is quickly frozen at a rate of about 3000 K/s to a temperature of -130 °C where it is maintained. The freezing of the sample is accomplished by blowing cold helium gas across the sample. The helium is cooled as it passes through a dewer filled with liquid nitrogen, and then is directed across the sample window to freeze the sample. Once the sample is frozen, it is able to withstand many exposures. In one experiment a cryogenically fixed 3T3 fibroblast cell was imaged 40 times in the same location (figure 1). At the resolution of this microscope there was no apparent change in the nuclear membrane due to the multiple exposures.

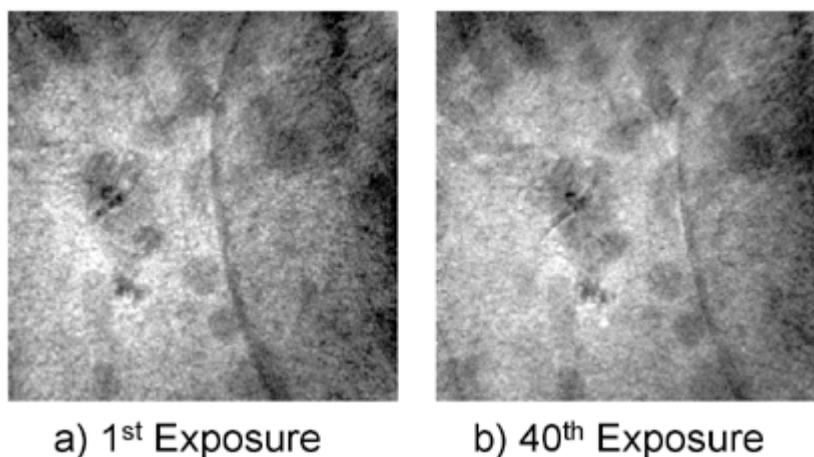


FIGURE 1. Cryogenically Fixed 3T3 Cells after a)1st and b)40th Exposures. The thin vertical line is the nuclear membrane which shows no observable modification due to the radiation dose. (with C. Larabell, D. Yager, unpublished)

In recent years many exciting scientific studies have been conducted making use of XM-1. In the biological arena, high-resolution protein localization has been demonstrated (6). Gold-labeled antibodies were used to selectively identify proteins and oligonucleotides within a cell.

The gold particles were silver-enhanced to form aggregates approximately 50 nm in diameter. These particles were resolved with the XM-1 and a mapping of the location of the target proteins and oligonucleotides were obtained. Some of the proteins and oligonucleotides labeled and imaged are microtubulin, nuclear pore complex, and actin mRNA. An example of the images is shown in figure 2, where tubulin protein labeled within a 3T3 epithelial cell is clearly seen. More recently, cryo-fixed images of a 3T3 cell were obtained as seen in figure 3 (6). In the montage assembly, the nuclear membrane, nucleoli, organelles, and granules are all clearly visible.

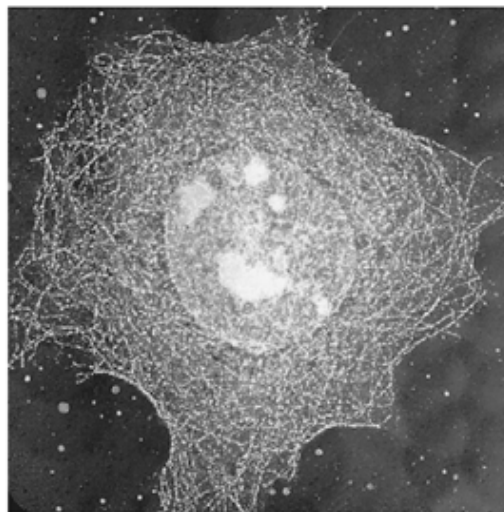


FIGURE 2. Tubulin Network in Epithelial Cell (C. Larabell, S. Lelievre, D. Hamamoto, M. Bissell, A. Nair and W. Meyer-Ilse, submitted for publication)

In addition, many material and environmental science studies were conducted using samples used in various water storage dams (7). The microscope has been used to study Alkali-silica reactions in concrete. These reactions can cause swelling and cracking of the dam structure. Reaction gel morphology was studied extensively with XM-1 (figure 4a). The microscope has also been used to observe the distribution of Mn in micronodules produced by biomineralization (8). Making use of the image contrast above and below the Mn L_3 edge provided the mechanism to produce a mapping of the Mn distribution. Moreover the high spatial resolution of the XM-1 was able to reveal a network of very fine needle like shaped structures.

The microscope was also used to study macromolecular structures of humic substances in aqueous solutions, soils, and in sediments (figure 4b) (9). These studies have shown that the macromolecular structures vary as a function of both solution chemical composition and mineral chemistry. This information is useful in accurately predicting the organo-mineral interactions, C-cycle, and contaminant transport in soils and aquatic systems. The above is just a sampling of studies using XM-1 during the past year. Additional studies involved nanocrystals, chromium-reducing bacteria, and magnetic materials.

Conclusion

A user-friendly, full field, x-ray transmission imaging microscope has been constructed and is in use for a wide variety of scientific studies at a spatial resolution of 36 nm. Users are able to obtain a set of images of their specimens, which can be dry, hydrated, or cryo-fixed. Plans are underway to improve the spatial resolution of the microscope and to develop its spectromicroscopic capabilities.

Acknowledgments

The authors would like to acknowledge our colleagues of the Center for X-ray Optics, the Life Science Division, and the ALS. In particular, the support from D. T. Attwood, D. Yager, and C. Larabell is to be noted. The US Dept. of Energy and The Office of Navy Research supported this work. AFOSR provided research support for student participation and training. This great instrument is a testament to the hard work, dedication, and insight of Werner Meyer-Ilse. His driving force and light will be deeply missed.

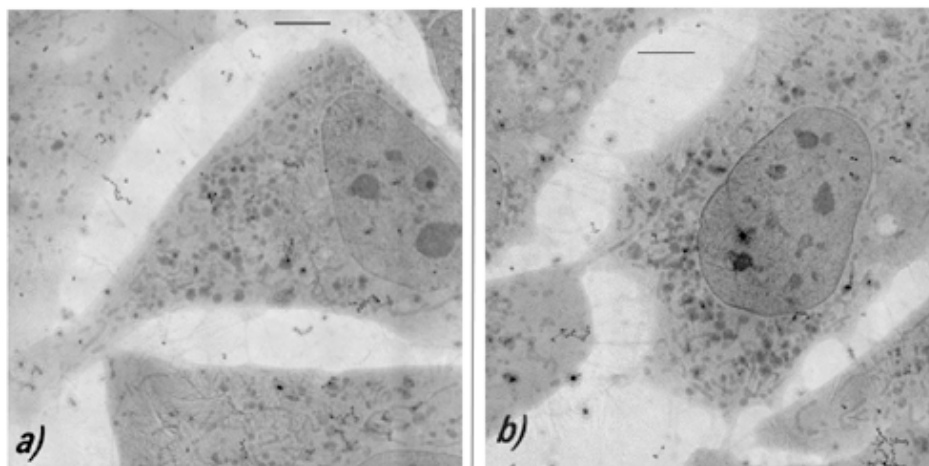


FIGURE 3. Cryogenically Fixed 3T3 Cells (with C. Larabell, D. Yager, T. Shin, XRM99 Proceedings)

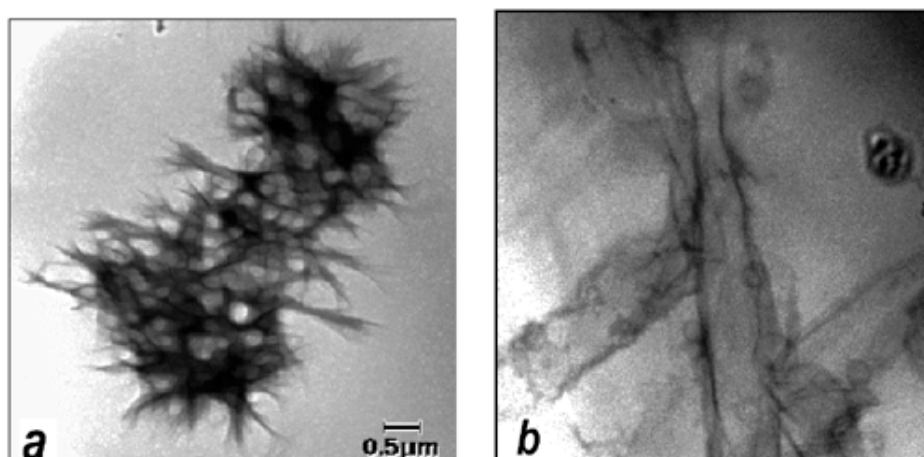


FIGURE 4. a) Alkali-silica reactions (K. Kurtis et al., ref 7) b) Humic substances in aqueous solutions (S. Myneni, et al, ref 9)

References

1. Meyer-Ilse, W., Medeck H., Jochum, L. Anderson, E., Attwood, D., Magowan, C., Balhorn, R., Moronne, M., Rudolph, D., Schmahl, G., *Synchrotron Radiation News* **8**, 23-33 (1995).
2. Schmahl, G., Rudolph, D., Niemann, B., Christ, O., *Quarterly Reviews of Biophysics* **13**, 297-315 (1980)
3. Anderson, E., Harteneck, B., Olynick, D., "Nanofabrication of X-ray Zone Plates with the Nanowriter Electron-Beam Lithography System", these proceedings
4. Underwood, J.H., Gullikson, E.M., " Beamline for measurements and characterization of multilayer optics for EUV Lithography", in *Emerging Lithographic Technologies II*, Proceedings of SPIE 3331, pp. 52-61.
5. Denbeaux, G., Johnson, L.E., Meyer-Ilse, W., "Spectromicroscopy at the XM-1", these proceedings
6. Larabell, C., Shin, T., Yager, D., "Localization of proteins and oligonucleotides using soft x-ray microscopy", these proceedings
7. Kurtis, K.E., Monteiro, P.J.M., Brown, J.T., Meyer-Ilse, W., "Investigation of Alkali-silica Reaction by Transmission Soft X-ray Microscopy", these proceedings
8. Rothe, J., Kneeder, E.M., Pecher, K., Tonner, B., Nealson, K.H., Grundl, T., Meyer-Ilse, W., Warwick, T., "Spectromicroscopy of Mn Distributions in Micronodules produced by Biomineralization"
9. Myneni, S.C.B., Brown, J.T., Martinez, G.A., Meyer-Ilse, W., "Imaging of humic substance macromolecular structures in water and soils", *Science Magazine*, in press.

Contact person: Gregory Denbeaux, Center for X-Ray Optics, Ernest Orlando Lawrence Berkeley National Laboratory. Email: gpdenbeaux@lbl.gov. Telephone: 510-486-4051.